Adaptive Focused Acoustics for the Formulation of Suspensions & Nano-Suspensions

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INTRODUCTION

The majority (~90%) of new chemical entities (NCEs) discovered by the pharmaceutical industry today are poorly soluble or lipophilic compounds; as are about 40% of existing drugs in the market. Consequently, this can create major challenges in drug development due to poor solubility, short biological half-life, poor bioavailability, prominent adverse effects, and stability of NCEs. Therefore, to evaluate these compounds at the preclinical stage, the compound is often dosed orally as an aqueous-based suspension, as a solution formulation may not easily be obtained without either toxic levels of excipients and/or considerable resources (i.e., impractical at an early stage when evaluating a high number of compounds). A potential downside to this approach is that dosing a suspension may have detrimental in vivo consequences such as decreased bioavailability and higher inter-subject variability when compared to dosing a solution formulation. A possible technique to mitigate this risk is reduction of the suspension’s particle size. However, there are few currently available methods to quickly reduce particle size across a range of sample volumes without introduction of potential contaminants due to the use of a reusable probe or degrading the API due to excessive heating. A novel technology, Adaptive Focused Acoustics™ (AFA™) (developed by Covaris Inc., Woburn, MA, USA) has been used to successfully reduce particle size in a controlled manner to make uniform suspensions with low micron or nano-scale particle sizes. This article describes how this controlled and broadly applicable technique is a scalable process that is more suitable over current methods at producing reduced particle size suspensions for achieving improved bioavailability and less variability in exposures.

CURRENT PROCESSES & LIMITATIONS

Micronization is employed to help address the low solubility issue by improving dissolution rate and its consequent bioavailability. The typical processes to formulate a simple suspension for preclinical oral dosing are sonication, homogenization, microfluidizers, stirring, and/or the use of excipients, such as the addition of surfactant wetting agents and polymers to promote homogeneity. A basic sonication bath can produce inconsistent results due to the unfocused and random nature of the sonic waves. These baths are limited in the peak power density achievable, and typically have “hot or cold spots.” Additionally, temperature-sensitive compounds are subject to heating in this process due to the need for high overall energy input to achieve the desired micronization effect. Mechanical homogenization is not ideal for small-scale volumes when compound is limited. It also promotes foaming in the formulation and makes cross-contamination a possibility. Additionally, operator to operator variability may be introduced. Like sonication, it can cause heating of temperature-sensitive compounds when used at higher intensity or for a significant amount of time. Microfluidizers produce very large...
amounts of heat and enable cross-contamination in the processing chamber. The sample must be cooled with a heat exchanger after processing. Additionally, the sample frequently must be passed through the system multiple times, and it is not uncommon to lose material in the process. Compounds may also be milled prior to formulation as an additional micronization step. This adds more time to the process and introduces loss of yield from the additional step. These techniques have issues, such as a broad size distribution in the drug particle produced, thermal degradation of the material, and contamination.

Wet milling, high-pressure homogenization, and microfluidizers are also used to produce nano-suspensions in-situ. The additional energy required in these processes exacerbates the issues mentioned above. Development of a proper formulation to stabilize the nano-suspension may be required. Limitations of these techniques related to the need for cleaning to avoid cross-contamination and/or a larger minimum volume needed to process material make it difficult to directly generate in-situ nano-suspensions with reasonable throughput for testing multiple iterations of formulations at a small scale.

**Adaptive Focused Acoustics:**

A more effective and versatile technique applicable to making suspension formulations of drugs with limited aqueous solubility is needed that overcomes all these limitations. A broadly based technology applicable to this class of molecule could have a tremendous impact on discovery effectiveness. The Covaris AFA technology is a self-contained, scalable, isothermal, and controllable process which is applied to generating reduced particle size suspensions of narrow distribution without degrading materials or allowing cross-contamination, and achieves 100% material recovery.

The Covaris AFA technology evolved from therapeutic lithotripsy (such as kidney stone treatment) and diagnostic imaging. The instruments developed by Covaris that incorporate AFA have wide-ranging applications from chemical compound management, DNA shearing for next generation sequencing methods, tissue disruption/homogenization, and formulation preparation. AFA works by sending convergent, high frequency, high intensity acoustic energy waves from a dish-shaped transducer (Figure 1). AFA is a form of mechanical energy. As acoustic/mechanical energy transfers through the sample, the material undergoes compression and rarefaction (expansion). At high intensity with fluid samples, this is typically embodied as cavitation events. Cavitation is the formation and subsequent collapse of bubbles. The acoustic energy applied to a sample causes bubbles to form from the naturally occurring dissolved gases and vapors of biological specimens and chemical fluids. When the energy is then removed, the bubble collapses. As the bubbles collapses, an intense, localized jet of solute (typically water) is created. This jet travels over a very short distance but at a very high velocity (> 100m/sec). As the number of bubbles is extremely high, the convergent energy density is very high, and the time interval is short (micro seconds), the consequent mixing (acoustic streaming) and/or disruption power capability of the process is substantial. A key point is the precise, reproducible control that is obtainable with the Covaris instrument systems utilizing AFA.

Similar to Covaris AFA, sonication is also an acoustic-based process. It has been used for a number of years in the life sciences industry; however, it is intrinsically distinct from Covaris AFA as it does not have the same level of control and reproducibility.
from AFA for a number of reasons. One key to the difference lies in the operating wavelength of each system. Sonication has a wavelength of 10’s of centimeters. This results in unfocused energy scattering, reflecting, and in many instances producing “hot spots”, which may readily damage some biological or chemical samples. By contrast, AFA wavelengths are short and focusable. This allows AFA to be both focused to a localized area of the sample and to be very efficient. For example, to achieve the identical internal pressure field in a sample, only 0.5 Watts of energy are required from a Covaris system, whereas over 80 Watts would be required from a sonicator system.

**MATERIALS & EQUIPMENT**

**PROCESSING EQUIPMENT**
- Covaris SF220 High Performance Formulation Processing System
- Parameters are controllable. Parameters for all processes mentioned: 300PIP, 50DF, 200C/B
- Net 150 Watts of power.

**PARTICLE SIZE INSTRUMENTATION**
- Nano particle range (Malvern Zetasizer Nano ZS-90)
- Micron particle range (Malvern Mastersizer 2000)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ibuprofen (1 mg/ml) (microns)</th>
<th>Ibuprofen (15 mg/ml) (microns)</th>
<th>Ibuprofen (100 mg/ml) (microns)</th>
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<tr>
<td>Baseline</td>
<td>203.67</td>
<td>203.67</td>
<td>203.67</td>
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<tr>
<td>2 ml, 5 min</td>
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<td>12 ml, 5 min</td>
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<td>18 ml, 10 min</td>
<td>39.53</td>
<td>34.04</td>
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**RESULTS & DISCUSSION**

Suspension formulations with particle size reduction from a controlled, broadly applicable technique are essential to achieving reproducible, high quality pharmacokinetic data at the preclinical stage. In these experiments, we demonstrate the ability for rapid particle size reduction in a generic suspension vehicle (0.5% methyl cellulose, 0.1% sodium lauryl sulfate) to a d(90) below 40 um for Ibuprofen, Cinnarizine, Indomethacin, and Griseofulvin at 15mg/ml. Ibuprofen concentration was then varied to demonstrate consistent results at 1mg/ml, 15mg/ml, and 100mg/ml. All of this was accomplished at three fixed volumes of 2ml, 12ml, and 18ml, which were chosen to encompass the volumes needed for early PK rodent dosing experiments. We then scaled up Ibuprofen to a homogenous 250ml suspension to demonstrate the scalability of using a flow cell without changing the mechanical attributes of the particle size reduction process.

Many new drug candidates originating from discovery programs are water insoluble with poor bioavailability, often leading to abandoning drug development efforts. The science of nano-suspensions is increasing the number of drug candidates that can be evaluated. Nano sized drug particles have a faster dissolution rate which can lead to faster...
or greater absorption. It is an effective and broadly applicable approach that goes beyond addressing water insolubility. Nano-particles can be used in tissue or cell specific targeting, have longer blood circulation capacity, greater stability against enzymatic degradation, and allow for the reduction of unwanted side effects. AFA was demonstrated to be highly effective at creating nano-suspensions, which will directly translate to an increase in the percentage of drug candidates viable for testing. In this experiment, a suspension vehicle (0.1% sodium lauryl sulfate, 0.025% methyl cellulose) was used with a 5mg/ml concentration of API processed in a 2ml vial for Ibuprofen, Cinnarizine, Indomethacin, and Griseofulvin. We demonstrate the ability to make low nanometer range suspensions by extending the processing times to 15 minutes. We then scaled up Cinnarizine to 250ml to demonstrate the scalability of using a flow cell for nano-suspension generation without changing the mechanical attributes of the particle size reduction process.

### Process Results (2-ml, 12-ml, 18-ml Batches)

The base line starting d(90) particle size for Ibuprofen is 203.667um with 97% of the particles above 40um. The samples were then processed for 5 minutes at 150 Watts under AFA. A 2ml vial (1mg/ml concentration) produced a d(90) population below 12.554um and 100% particles below 20um. A 12ml vial (1mg/ml concentration) produced 33.353um (d90), and 97% of the particles are below 40um. In the case of an 18ml vial, it took 10 minutes for 97% of the particles to get to below 40um. Concentrations were increased to both 15mg/ml and 100mg/ml of Ibuprofen; in 5 minutes of processing, the d(90) was below 40um for the 2ml and 12ml vials, and with 10 minutes processing, the 18ml vials had d(90) populations below 40um. These results were repeated for both Indomethacin and Cinnarizine at 15mg/ml, with slight variations in size distributions. In the case of Griseofulvin at 15mg/ml, the starting d(90) particle size was approximately 40um. The d(90) particle size was brought below 20um in 5 minutes for the 2ml and 12ml vials, and 10 minutes for the 18ml vial. The particle size results for the four compounds at 15mg/ml are listed in Table 1. Table 2 lists the particle sizes for Ibuprofen at 1mg/ml, 15mg/ml, and 100mg/ml. Figure 2 illustrates before and after processing of 15mg/ml Ibuprofen at the 12ml volume.

### Generic Scale-Up for Ibuprofen: 250-ml Batch

The base line particle size of the Ibuprofen is d(90) 203.667um and d(50) 97.834um, and almost 95% of the particles are above 40um. Scaling up to a volume of 250ml at a flow rate of 30ml/min at 15 minutes produced 90% of the particles below 40um; d(90) 39.726um, d(50) 21.701um, and d(10) 6.805um. At 30 minutes, 99.37% of the population are below 40um; d(90) 31.091um, d(50) 16.167um, and d(10) 3.583um. At 60 minutes, 99.39% of the particles are below 40um; d(90) 28.005um, d(50) 14.843um, and d(10) 3.351um. Therefore, assuming a linear conversion ratio where 2ml is scaled up to 250ml, it should take 10.41 hours to attain < 40um particles. In practice, it required 15 minutes to achieve the desired result, thus demonstrating a favorable scaling factor over 40 times more efficient when processing the higher volume of material. Figure 3 illustrates this particle size reduction over time. Homogeneity and stability was demonstrated by sampling from the 250ml suspension at the top, middle, and bottom depths. The suspension aliquots were analyzed by HPLC.

### Table 3

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>Cinnarizine</th>
<th>Indomethacin</th>
<th>Griseofulvin</th>
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<tbody>
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<td>Baseline</td>
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<td>44.45 microns</td>
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<tr>
<td>15 minutes</td>
<td>110 nm</td>
<td>280 nm</td>
<td>127.4 nm</td>
<td>100 nm</td>
</tr>
<tr>
<td>30 minutes</td>
<td>97 nm</td>
<td>56.85 nm</td>
<td>20 nm</td>
<td>90 nm</td>
</tr>
</tbody>
</table>

**2-ml Nano-suspension, Average Particle Size 5 mg/ml**

**FIGURE 4**

Cinnarizine 250ml 5mg/ml

0.1% SLS 0.025% MC

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**250-ml nano-suspension, 15-mg/ml Cinnarizine particle size reduction over time.**
using a stability-indicating method as a guide. At the first measured time point of only 15 minutes, the samples showed that the suspension was homogenous, having a relative standard deviation of only 0.40%. This was maintained through 60 minutes, where the samples had an RSD of 0.38%. The Ibuprofen was chemically stable, showing no impurity growth over the 60 minutes of processing.

**Nano-Suspension Process Results (2-ml Batches)**

In 15 minutes, nano-suspensions were generated with an average particle size ranging from 100 - 280nm and at 30 minutes, a range of 20 - 97nm was achieved. Results are listed in Table 3. The SF220 enables generation of nano-particles and the practical screening of potential formulations to stabilize them in the same step at small scale without the uncontrolled heating, sample loss, and/or higher volume requirement of other nano-suspension generation techniques. This saves time and eliminates compound waste.

**Nano-Suspension Scale-Up for Cinnarizine (250-ml Batch)**

Following 1 hour of processing, a 250ml suspension (which started at 200um), a 1um particle size was achieved, and by 9 hours, it stabilized at approximately 200nm. The suspension vehicle used was 0.1% sodium lauryl sulfate, 0.025% methyl cellulose in water. The surfactant concentration was significantly below the CMC range. Figure 4 illustrates this particle size reduction over time.

**CONCLUSION**

Adaptive Focused Acoustics (AFA) technology enables an instrument that capably and effectively results in reproducible suspension formulations at both the micron and nano-scale size range. Routine, high throughput preclinical formulation efforts aimed at screening early stage compounds in PK studies can thus be completed in a self-contained, controlled, and partially automated fashion. In this area of preclinical formulation, use of the Covaris SF220 system will improve the overall quality of experiments by reducing formulation preparation errors and dosing variability, while offering rapid, standardized protocols to reduce particle size. Formulation development is enhanced with a novel tool that allows for faster results with less material that is scalable.

**REFERENCES**

6. Wilhelm D., 2010 Development and validation of an HPLC method to analyze ibuprofen and impurities according to the European Pharmacopoeia. Agilent Technologies publication number 5989-9241EN.